EFFECT OF MILKY DISEASE ON *TIPHIA* PARASITES OF JAPANESE BEETLE LARVÆ

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INTRODUCTION

Several species of *Tiphia* have been introduced from the Orient in the fight against the Japanese beetle, *Popillia japonica* Newm., and two have become particularly well established throughout the older beetle-infested area. One of these, *Tiphia vernalis* Roh., has its active flight period during the spring months, whereas the other, *Tiphia popilliavora* Roh., is present late in the summer. Large collections of adult *Tiphia* females are made annually from a number of these colonies for further use in the colonization of other infested areas. The abundance of *Tiphia* in a number of the colonies ecologically similar to the more successful ones is not sufficient to warrant collection, and in some of these no satisfactory explanation of this condition has been offered. It seemed possible that milky disease, caused by *Bacillus popilliæ* Dutky, in competition with *Tiphia* might be responsible for the failure to obtain strong colonies in areas where this disease was prevalent. Work was started in 1935 by the writer under the direction of the late G. F. White and was continued through 1937, both in the laboratory and in the field, to determine whether the milky disease does kill *Tiphia* larvæ that feed on diseased hosts, and to clear up some of the questions on the interrelationships of these two biological factors when they occur in the same habitat.

EXPERIMENTAL PROCEDURE

In attempting to solve this problem it was essential to start with Japanese beetle larvæ that were known to be healthy. Larvæ were therefore collected in the field and held individually in 2-ounce tins of autoclaved soil for 3 weeks at a temperature of 75° F., and those not showing signs of disease at the end of this period were assumed to be healthy. Likewise, as *Tiphia* that had

not been exposed to the milky disease organisms were required, females only recently emerged from laboratory experiments were mated and used in these studies.

Experiments were conducted in which larvæ parasitized by *Tiphia* females were inoculated with the disease organism at different periods of development to determine whether the parasite would develop on diseased larvæ. The inoculation of the host larvæ was accomplished by simply puncturing them with a needle previously dipped in the blood of diseased larvæ. In some of these experiments the larvæ were inoculated and parasitized on the same day, while others were inoculated from 2 to 15 days prior to parasitization. Other series were conducted in which the host larvæ were inoculated from 2 to 15 days following parasitization. Still other larvæ were parasitized and held in infectious soil for a varying number of days prior to cocoon formation. Similar experiments were also conducted in 1936 with both *T. popilliavora* and *T. vernalis*.

RESULTS

In some of the earlier experiments *Tiphia* larvæ examined immediately after they had spun cocoons were found to be infected with milky disease. Other cocoons from these same series, examined 2 weeks later, showed no signs of disease in the enclosed larvæ. Further investigation showed that the recently voided meconium present in the posterior end of the cocoons was often laden with viable spores of the agent causing milky disease. It was found that after the meconium had been voided there was no evidence of disease in the larva, indicating that the spores had been voided along with the meconium, with no ill effect on the parasite itself.

Table 1 gives a summary of the results obtained in examinations of *Tiphia vernalis* cocoons during 1935 and 1936, including only cocoons in which the meconium was examined. In this work the meconium is spoken of as being positive if it was found to contain spores of the milky disease.

From these data it seems unlikely that *Bacillus popilliæ*, the organism causing milky disease, has any effect on the mortality of *Tiphia* larvæ within the cocoon, as 74 per cent of the larvæ within cocoons formed in the check experiments were dead after

2 months, as compared with 52 per cent of those formed in experiments in which the hosts were exposed to the disease agent. Also, only 3 per cent of the cocoons containing dead larvæ in the checks contained positive meconium, as compared with 36 per cent of those exposed to disease, indicating that the presence of the milky disease agent was not the direct cause of mortality.

In one series of experiments, all host larvæ inoculated with milky disease 10 and 14 days prior to parasitization by *Tiphia* died before the parasite larvæ could complete their development.

TABLE I

Summary of *Tiphia vernalis* Cocoon Examinations Made 2 Months After Cocoon Formation, 1935 and 1936

Treatment of host larvæ	Cocoons formed	Cocoons containing dead *Tiphia* larvæ	Cocoons of dead *Tiphia* containing positive* meconium
	Number	*Per cent*	*Per cent*
Exposed to disease	181	52	36
Not exposed to disease	104	74	3

* Containing spores of *Bacillus popilliæ*.

However, only 37 per cent of such larvæ inoculated 2 days after parasitization died before the parasite larvæ completed development and spun cocoons. Although excessive mortality of the host larvæ caused directly by milky disease occurred in these experiments, a large percentage of the cadavers when examined still had living *Tiphia* larvæ attached to them, indicating that ultimate death of the parasite was due to lack of a living host and not to the direct effect of the disease on the parasite.

Experiments comparable to those previously described were conducted with *Tiphia popilliavora* during the fall of 1936. A portion of the cocoons from these experiments were set aside for possible emergence. Examination of a large number of the cocoons 30 days after formation (Table 2) showed that approximately the same mortality of enclosed *T. popilliavora* larvæ occurred, regardless of whether the hosts had been exposed to the disease.

As only a small series of cocoons was held in storage, very little emergence resulted. Emergence of both male and female *Tiphia*

was obtained, however, and examination of the meconium remaining in the cocoons after emergence showed the presence of milky disease spores. This is conclusive proof that *Tiphia* parasites can and do complete development on, and emerge from, host larvæ infected with milky disease, provided the host does not die before the parasite completes its development on it.

Some evidence that *Tiphia* may aid in the spread of milky disease was observed during the course of these experiments. *Tiphia*

TABLE II

SUMMARY OF *Tiphia popilliavora* COCOON EXAMINATIONS 30 DAYS AFTER COCOON FORMATION, 1936

Treatment of host larvæ	Cocoons formed	Cocoons containing dead *Tiphia* larvæ	Cocoons of dead *Tiphia* containing positive* meconium
	Number	*Per cent*	*Per cent*
Exposed to disease	142	42	40
Not exposed to disease	104	45	4

* Containing spores of *Bacillus popilliæ*.

adults were removed at various times from infectious soil and examined externally for disease spores. From a total of 71 examinations, 22 *Tiphia* adults were demonstrated to be carriers of spores.

A number of well-established colonies of *Tiphia* occur in areas in which milky disease is also present. Surveys conducted at several of these sites have been made and data from one such survey are given below. Newly formed cocoons found at the time of this survey were cut open and an examination made of the voided meconium to determine whether the respective hosts had been diseased. An average population of 12 Japanese beetles, including all stages, per square foot was found, as follows:

Number of holes dug	36
Total population (includes larvæ, adults, and pupæ of the Japanese beetle and *Tiphia* cocoons)	434
Total hosts parasitized	210 (48.4 per cent)
Total hosts diseased	167 (38.5 per cent)
Hosts both parasitized and diseased	63 (14.5 per cent)
Hosts neither parasitized nor diseased	120 (27.6 per cent)
Total hosts killed either by disease or parasitization	314 (72.4 per cent)

It is evident that there was an overlap of 14.5 per cent between hosts that were parasitized and those that were diseased, *i.e.*, 63 hosts were parasitized as well as diseased. This condition was in effect superparasitism, because death of the host would have resulted from either biological agency. The combination of disease and parasitization at this location accounted for 72.4 per cent of the total population present during June 1936. In the examination of the cocoons from this survey it was found that 8 of the cocoons containing dead *Tiphia* larvæ and 2 of those containing living parasite larvæ had meconium with viable milky disease spores. The larvæ in the last two cases had successfully voided the spores with no apparent ill effect. In no case after a living *Tiphia* larva had voided its meconium were milky disease spores found in the larva itself.

CONCLUSIONS

From observations both in the field and in the laboratory, it is evident that some of the progeny of *Tiphia* do fail to complete their development, owing to the death of the host through disease but not to the disease directly. The greatest loss of hosts occurs when the disease is well advanced at the time of parasitization. On the other hand, during May, when *Tiphia vernalis* is actively ovipositing, the soil temperature in the Moorestown, N. J., area rarely exceeds 65° F., a temperature not favorable for the rapid growth of the disease organism. The ability of *T. vernalis* to persist in such areas is borne out further by the fact that this species may still be collected by the thousands in areas in which the incidence of disease has been rather consistently high since 1936. It seems more probable that a species such as *T. popilliavora,* which is active in the latter part of August, will suffer most, because at that time the soil temperature ranges generally somewhat above 70° and is more favorable to disease development.

It is not likely that either of these biological agents can completely eradicate its host. Both will therefore persist in varying degrees, and no doubt some *Tiphia* colonies, which might otherwise have become sufficiently populous to serve as collecting centers, will suffer reductions in population owing to the effect of the disease on the host. It is also possible that *Tiphia,* acting as

a vector of viable spores, may be assisting in the dispersal of the disease.

As data conclusively show that *Tiphia* parasites can complete their development on hosts infected with milky disease, it is the writer's opinion that these two biological agencies are compatible as control factors within the same area.